BIOPROSPECTS OF CULTURABLE LOW-DENSITY POLYETHYLENE DEGRADING BACTERIA INHABITING IN DUMPING SITES OF JAIPUR, RAJASTHAN

RESEARCH PLAN

FOR REGISTRATION IN DOCTOR OF PHILOSOPHY

By

HEMLATA SHARMA

Under the Supervision of

Dr. Deepesh Kumar Neelam
Assistant Professor
Department of Microbiology

JECRC UNIVERSITY
2020

FACULTY OF SCIENCE
JECRC UNIVERSITY
2020

CERTIFICATE

This is to certify that this Research Proposal entitled **"Bioprospects of Culturable Low-Density Polyethylene Degrading Bacteria Inhabiting in Dumping Sites of Jaipur, Rajasthan"** by **Hemlata Sharma,** submitted in fulfilment of the requirement for registration in Ph.D. program in **Department of Microbiology** under Faculty of Sciences of JECRC University, Jaipur, during the academic year 2020, is a bonafide record of work carried out under my guidance and supervision.

Date: 18/06/2020

Dr. Deepesh Kumar Neelam
Assistant Professor
Department of Microbiology
JECRC University
Jaipur

S.N.	CONTENT	PAGE NO.
1	Introduction	1
2	Literature Review	2-3
3	Description of Broad Area/Topic	3-4
4	Objectives of Study	4
5	Methodology to be Adopted	5-6
6	Expected Outcome of the Research	6
7	References	7-8

Research Plan

ORIGINALITY REPORT

6%	2%	2%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Kenyatta University Student Paper	3%
2	ijpbs.net Internet Source	2%

Exclude quotes	On	Exclude matches	< 14 words
Exclude bibliography	On		

INTRODUCTION

The plastic demand is tremendously increasing day by day all over the world at an extremely high rate. The cause behind this increase is the physical and chemical properties like durability, lightness, hydrophobicity and strength which makes it better packaging material over the other packaging materials like cellulose and paper based products (Padmanabhan et al., 2020). Among synthetic polymers, Low-Density Polyethylene (LDPE) is widely used as a packaging material for food, agricultural products, electronic wares and vehicles etc. Biodegradable plastics have been made as an alternative to synthetic plastic but cannot be used widely as it is not always degradable under natural conditions and even can't be recycled as synthetic plastics do (Veethahavya et al., 2016). LDPE biodegradation is area of concern for researchers across the world as the polyethylene, we used is very harmful for the environment and cause environmental pollution.

Biodegradation of plastic was observed by microorganisms (either by bacteria or by fungi). The degradation depends upon the location where polyethylene is dumped it may be soil, landfils or water (lakes, ponds & rivers). It also depends upon the chemical properties of LDPE and the microorganisms involved in degradation (Padmanabhan et al., 2020). The biodegradation can be either aerobically or an aerobically. In the condition of aerobic degradation it was observed that CO_2 and H_2O were produced and by anaerobic degradation CO_2, H_2O and CH_4 were produced (Shah et al., 2008). Polymers are broken into monomers during biodegradation process and degradation takes place inside the microbial cells (Padmanabhan et al., 2020). Pretreatment such as theromo-oxidative degradation (heat+oxygen) and photo degradation (UV-light) will further enhance the degradation process. It creates carbonyl, ester and carboxyl functional group which help in the reduction of hydrophobicity (Devi et al., 2015). Pretreated polyethylene showed reduction in it's molecular weight so as to make it easier for microorganisms to degrade it further (Shah et al., 2008). It was observed that the polymers containing side chains are difficult to degrade comparison to the linear polymer (Padmanabhan et al., 2020).

At present, only less number of studies showed biological degradation of plastic. So, the proposed study aims to isolation and characterization of potential plastic (LDPE) degrading bacterial diversity from Mathuradaspura and Langadiyawas dumping sites of Jaipur, Rajasthan.

LITERATURE REVIEW

1. In 2013, Carson his colleagues investigated the relationship between plastic associated microorganism communities and factors such as location, temperature, salinity, plankton abundance, plastic concentration, item size, surface roughness and polymer type. Small plastic items from North Pacific Gyre were examined and concluded that *Bacillus* bacteria and pennate diatoms were most abundant (Carson et al., 2013).

2. Two bacterial strains namely *Enterobacter asburiae* and *Bacillus* sp., capable of degrading polyethylene were isolated from the gut of Waxworm or Indian mealmoths (Yang et al., 2014).

3. In the search of platic degrading bacteria another study was focused on the degradation of LDPE by *Acinetobacter baumannii* (Pramila and Ramesh, 2015).

4. Researchers also worked on the plastic degrading ability of *Aspergillus oryzae* under ex-situ conditions (Indumathi et al., 2016).

5. Another research showed the degradation ability of *Bacillus subtilis*, utilizing the polythene as their sole carbon source and also produce surface active compound i.e. surfactin (biosurfactant) that enhance the degradation process (Vimala and Mathew, 2016).

6. This study reported that the obtained isolates from Mangrove environment of Gupta para, South Andaman not able to produce L-Asparaginase enzyme but also showed plastic degrading activity (Manoj et al., 2018).

7. A study suggested that there are many microorganisms like *Acinetobacter, Pseudomonas, Micrococcu*s and *Sphingobacterium* etc. present in natural habitat that are able to degrade polyetylene plastic. A combination of photo-oxidation induced by UV-radiation exposure and biodegradation with new bacteria may improve plastic degradation in natural and feasible way without any ecological impacts (Montazer et al., 2018).

8. Another research demonstrated that *Cupriavidus necator* H16 produce short length polyhydroxyalkanoate polymer while *Pseudomonas putida* LS46 and *Acinetobacter pitti* IRN19 produced medium chain length polyhydroxyalkanoate while growing on polyethylene

powder. This was the first report that provided the direct evidence for simultaneous bioconversion of LDPE plastic to biodegradable polyhydroxyalkanoate polymers (Montazer et al., 2019).

9. This study presented both aspects of microbial ecotoxicology on marine plastic debris, namely the impacts of plastic on marine microbial life and inversely how microbes can play a role in plastic biodegradation (Jacquin et al., 2019).

10. Recently study suggested that *Bacillus* spp. and *Pseudomonas* spp. are more efficient for degradation of HDPE and can be used as cost-effective and safe approach for elimination of plastic waste (Devi et al., 2019).

11. Padmanabhan and his colleagues explained that the microbial species are isolated from different sources like cooking oil, grease and petroleum production. Two bacterial species *Sphingomonas, Pseudomonas aeruginosa* and three fungal species such as *Aspergillus niger, Aspergillus falvus* and one unidentified fungal species were isolated from these sources, used for the degradation of polyethylene plastic samples (Padmanabhan et al., 2020).

DESCRIPTION OF TOPIC

Plastics are man- made synthetic polymers that are used in day to day life. It has wide range of industrial applications. There are various synthetic plastics which are commercially used such as polyethylene, polyvinylchloride, polystyrene, polyethylene terephthalate, polypropylene and polyurethanes etc. and among these polyethylene (low density polyethylene) is widely used as a packaging material (Devi et al., 2015; Raziyafatima et al., 2016). Plastics are used extensively due to it's low cost (Raziyafatima et al., 2016). But the major problem associated with plastic is there safe disposal an degradation. Plastic accumulating at a very high rate but the degradation process is very slow. It adversely affects the wild life, equatic ecosystem, the land and humans as well (Devi et al., 2015). The degradation refers to change in physical and chemical properties due to the effect of light, heat or biological activity (Shah et al., 2008). There are basically two types of plastics, degradable and non-degradable. The synthetic plastics are non-degradable due to it's high molecular weight then the degradable plastics (Raziyafatima et al., 2016). Various researchers are focussing on degradation of polyethylene through green approach or eco-friendly approach so as to minimise environmental pollution.

As a plastic, polyethylene cannot degrade easily; it will lead to environmental pollution. The entire world is facing this problem. So, in the present study the main aim is try to overcome the plastic (LDPE) degradation problem by biological process using bacteria. This study will increase the scientific information for society and provide a platform for entrepreneurs to make their effort in the field of environmental waste management.

OBJECTIVES OF THE STUDY

- To collect the soil samples from solid waste dumping sites near Jaipur and physico-chemical characterization of sampling sites
- To isolate and characterization of bacteria
- To screen the isolated bacteria which may able to degrade low-density polyethylene
- To identify only potential low-density polyethylene degrading bacteria using 16S rRNA gene to evaluate novelty and phylogeny
- To study the polyethylene biodegrading efficiency of selected bacterial isolate

STUDY AREA

Jaipur is the capital city of Rajasthan covering geographical area of about 480 square kilometer (sq km). The solid waste management of Jaipur city is done by Jaipur Municipal Corporation. The location of the both study area are as follows

1. Mathuradaspura

This site is located 17 km away in the east direction from the Jaipur city area at 26º57’10” North latitude and 75º 55’54” East longitudes.

2. Langadiyawas

It is also located in the east direction of the city at 26º35’3” North latitude and 76º 30’5” East longitudes. This site is about 3-4 km away from the Mathuradaspura.

METHODOLOGY

The following methodology would be undertaken to carry out the present work

1. Soil samples collection- Soil samples will be collected with the help of sterile spatula and transfer in to sterile plastic bags or sterile sample collection bottles from the soil depths of 10 cm.

2. Analysis of physicochemical characteristics of sampling sites- Physicochemical conditions during sampling will be determined at the sampling site by thermometer and pH strips. Other physicochemical characteristics (TDS, Salinity and Conductivity) will be determined in the laboratory (Bundela et al., 2012).

3. Isolation, microscopic examination and characterization of bacteria- Soil samples will inoculate in culture media and incubate at 37 °C for 24-72 hrs. The pure single colony of individual microorganism will isolate by serial dilution method followed by (A).streak plate method (B). spread plate method. Pure single colony isolates culture will maintain in glycerol solution or in mineral oil for further research study (Begum et al., 2015).

4.Microscopic examination- Morphological studies of bacterial colonies on the basis of their colour, size, shape, margins and pigmentation will be examined under microscope. Bacterial colonies of different samples will be identifying by Gram's Staining method (Begum et al., 2015).

5.Biochemical characterization- Biochemical characterization of bacterial isolates will perform by various biochemical tests like indole test, MR-VP test, simmons citrate, starch hydrolysis, H_2S production, catalase, oxidase, urease sugar fermentation and nitrate reduction test (Begum et al., 2015).

6. Screening of low-density polyethylene degrading potential isolates- Isolated bacteria will be further observed for its plastic degrading ability. LDPE will be added into mineral salt medium at a concentration of 0.1%(w/v) respectively. Mixture will be sonicated for 60 minutes at 120 rpm and will further autoclave at 121 °C and pressure of 15 lbs/inch^2 for 20 minutes. Isolated bacteria will inoculate on nutrient agar and incubated at 25-30 °C for 2-4 weeks (Usha et al., 2011).

7. Molecular identification of selected isolates- Potential isolates will be confirmed identify by 16S rRNA sequencing and accurate position of each isolate will be define by phylogenetic tree (Harshvardhan and Jha , 2013).

8. Polyethylene biodegrading efficiency of isolates in pure culture- Efficiency of plastic degradation will be performed in liquid medium culture media with optimum parameters. All expected results will be analyzed by dry weight of residual polyethylene, Biofilm formation, Fourier-Transform infrared spectroscopy (FT-IR), Bacterial cell surface hydrophobicity, Scanning electron microscopy etc. (Harshvardhan and Jha, 2013; Balasubramanian et al., 2010).

EXPECTED OUTCOME

- This study could be provide the information about separation of plastic degrading novel bacterial strains in comparison of non plastic degrading bacteria isolated from same dumping sites.

- This study will help to provide economically valuable procedure for plastic biodegradation by biological method using bacteria.

REFERENCES

1. Padmanabhan L., Varghese, S., Patil, R.K., Rajath, H.M., Krishnasree, R.K. and Shareef, M.I (2020) *"Ecofriendly Degradation of Polyethylene Plastics Using Oil Degrading Microbes"*. Recent Innovations in Chemical Engineering (Formerly Recent Patents on Chemical Engineering), 13(1), pp.29-40.

2. Veethahavyaa, K.S., Rajath, B.S., Noobiab, S. and Kumar, B (2016) *"Biodegradation of low density polyethylene in aqueous media"*. Procedia Environmental Sciences 35: 709–713.
3. 3.Shah, A.A., Hasan, F., Hameed, A. and Ahmed, S (2008) *"Biological degradation of plastics: a comprehensive review"*. Biotechnology advances, 26(3), pp.246-265.
4. Devi, R.S., Kannan, V.R., Nivas, D., Kannan, K., Chandru, S. and Antony, A.R (2015) *"Biodegradation of HDPE by Aspergillus spp. from marine ecosystem of Gulf of Mannar, India"*. Marine pollution bulletin, 96(1-2), pp.32-40.
5. Carson, H.S., Nerheim, M.S., Carroll, K.A. and Eriksen, M (2013) *"The plastic-associated microorganisms of the North Pacific Gyre"*. Marine pollution bulletin, 75(1-2), pp.126-132.
6. Yang, J., Yang, Y., Wu, W.M., Zhao, J. and Jiang, L (2014) *"Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms"*. Environmental science & technology, 48(23), pp.13776-13784.
7. Pramila, R. and Ramesh, K.V (2015) *"Potential biodegradation of low density polyethylene (LDPE) by Acinetobacter baumannii"*. African Journal of Bacteriology Research, 3(1), pp.92-95.
8. Indumathi A, Gayathri, T., Biotechnology, E., Nadu, T. and Nadu, T (2016) *"Plastic Degrading ability of Aspergillus oryzae isolated from the garbage dumping sites of Thanjavur, India"*. International Journal Current Microbiology Applied Sciences 8(13), pp.2319-7706.
9. Vimala, P.P. and Mathew, L (2016) *"Biodegradation of Polyethylene using Bacillus subtilis"*. Procedia Technology, 24, pp.232-239.
10. Manoj, B., Ramesh, C.H. and Mohanraju, R (2018) *"Isolation of plastic degrading and L-Asparaginase Enzyme Producing Bacteria from the Mangrove Environment of Guptapara, South Andaman"*. International Journal for Research in Applied Science & Engineering Technology, 6, pp.29-36.

11. Montazer, Z., Habibi-Najafi, M.B., Mohebbi, M. and Oromiehei, A (2018) *"Microbial degradation of UV-pretreated low-density polyethylene films by novel polyethylene-degrading bacteria isolated from plastic-dump soil"*. Journal of Polymers and the Environment, 26(9), pp.3613-3625.
12. Montazer, Z., Habibi Najafi, M.B. and Levin, D.B (2019) *"Microbial degradation of low-density polyethylene and synthesis of polyhydroxyalkanoate polymers"*. Canadian journal of microbiology, 65(3), pp.224-234.
13. Jacquin, J., Cheng, J., Odobel, C., CONAN, P., Pujo-pay, M. and Jean-Francois, G (2019) *"Microbial ecotoxicology of marine plastic debris: a review on colonization and biodegradation by the plastisphere"*. Frontiers in microbiology, 10, p.865.
14. Devi, R.S., Ramya, R., Kannan, K., Antony, A.R. and Kannan, V.R (2019) *"Investigation of biodegradation potentials of high density polyethylene degrading marine bacteria isolated from the coastal regions of Tamil Nadu, India"*. Marine pollution bulletin, 138, pp.549-560.
15. Raziyafathima, M., Praseetha, P.K. and Rimal, I.R.S (2016*) "Microbial degradation of plastic waste: a review"*. Chemical and Biological Sciences, 4, pp.231-42.
16. Bundela, P.S., Sharma, A., Pandey, A.K., Pandey, P. and Awasthi, A.K (2012) *"Physicochemical analysis of ground water near municipal solid waste dumping sites in Jabalpur"*. International Journal of plant, animal and environmental sciences, 4(1), pp.217-222.
17. Begum, M.A., Varalakshmi, B. and Umamagheswari, K (2015) *"Biodegradation of polythene bag using bacteria isolated from soil"*. International Journal Current Microbiology Applied Sciences , 4(11), pp.674-680.
18. Usha, R., Sangeetha, T. and Palaniswamy, M (2011) *"Screening of polyethylene degrading microorganisms from garbage soil".* Libyan Agriculture Research Center Journal International, 2(4), pp.200-4.
19. Harshvardhan, K. and Jha, B (2013) "*Biodegradation of low-density polyethylene by marine bacteria from pelagic waters"*, Arabian Sea, India. Marine Pollution Bulletin, 77(1-2), pp.100-106.
20. Balasubramanian, V., Natarajan, K., Hemambika, B., Ramesh, N., Sumathi, C.S., Kottaimuthu, R. and Rajesh Kannan, V (2010) *"High-density polyethylene (HDPE)-degrading potential bacteria from marine ecosystem of Gulf of Mannar, India"*. Letters in applied microbiology, 51(2), pp.205-211.